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(54) Title: ADDUCTS BETWEEN MAGNETIC RESONANCE SHIFT REAGENTS AND SUBSTRATES CONTAINING EXCHANGEABLE PROTONS FOR "CEST" APPLICATIONS

(57) Abstract: Disclosed are CEST paramagnetic agents comprising a substrate (SH) containing mobile protons bonded to a paramagnetic chelate (SR) containing a metal selected from iron (II) (high-spin configuration), iron (III), cobalt (II), rhodium (II), copper (II), nickel (II), cerium (III), praseodymium (III), neodymium (III), dysprosium (III), erbium (III), terbium (III), holmium (III), thulium (III), ytterbium (III) and europium (III).

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ADDUCTS BETWEEN MAGNETIC RESONANCE SHIFT REAGENTS AND SUBSTRATES CONTAINING EXCHANGEABLE PROTONS FOR "CEST" APPLICATIONS

The present invention relates to paramagnetic adducts useful in Magnetic Resonance Imaging (MRI) procedures which use saturation transfer to create image contrast.

### Background to the invention

5       The generation of contrast in an MRI image through irradiation of mobile protons in the tissues has already been reported in US 5,050,609. The use of exogenous contrast agents (originally called CEDST agents, and now called CEST agents) containing at least one mobile proton in exchange with water was disclosed in WO 00/66180. That document does not expressly refer  
10 to the possibility of using paramagnetic complexes as CEST agents. The preferred compounds are diamagnetic molecules such as sugars, aminoacids, heterocyclic compounds, nucleosides, imidazoles and derivatives, guanidine, etc.

15       The same document also claims methods for *in vitro* and *in vivo* determination of the pH, temperature and metabolite concentration by administering a contrast agent having two sets of magnetically non-equivalent mobile protons. Selective irradiation of the two pools and application of a ratiometric method allows a transfer effect to be measured independently of the concentration of contrast medium. Saturation transfer implies that the  
20 mobile protons of the CEST agent do not coalesce with the bulk water, which means that the separation between the resonance frequencies of the two sets of mobile protons must be greater than the exchange rate. Moreover, the extent of the transfer is directly proportional to the exchange rate of the protons of the agent, which means that an increase in separation of the resonance

frequencies of the two exchanging signals increases the efficiency of saturation transfer. The use of CEST paramagnetic contrast agents was therefore considered in WO 02/43775, which claims the use of paramagnetic complexes having mobile protons whose saturation transfer (which is better  
5 than that of the diamagnetic compounds exemplified in US 5050609) can be sensitive to parameters of diagnostic interest such as pH, temperature, metabolite concentration, etc.

However, the possibility of using as CEST agents systems constituted by non-covalent adducts formed by a substrate having protons in exchange  
10 with bulk water (including types similar to those exemplified in US 5050609) and by a paramagnetic complex able to increase the separation of their resonance frequencies off the bulk water is not considered in the above-mentioned documents or in the literature. With this approach, the paramagnetic agent will be chosen on the basis of its Shift Reagent (SR)  
15 characteristics in relation to the properties of the compound containing the exchangeable protons.

### **Description of the invention**

The present invention relates to the preparation and use of paramagnetic CEST agents wherein the chemical shift of the mobile protons which must be  
20 irradiated to observe saturation transfer has been suitably "shifted" as a result of interaction of the substrate with a paramagnetic chelate containing a metal selected from iron (II) (high-spin configuration), iron (III), cobalt (II), rhodium (II), copper (II), nickel (II), cerium (III), praseodymium (III), neodymium (III), dysprosium (III), erbium (III), terbium (III), holmium (III),  
25 thulium (III), ytterbium (III) and europium (III). The mobile protons can belong to diamagnetic or paramagnetic substrates, provided that in the latter case their chemical shift is influenced by non-covalent interaction with the paramagnetic species.

The first aspect of the invention consequently relates to paramagnetic CEST agents constituted by diamagnetic substrates containing mobile protons (SH units) bonded by means of electrostatic interactions or covalent bonds to paramagnetic chelates (SR units) which interact reversibly with the substrates.

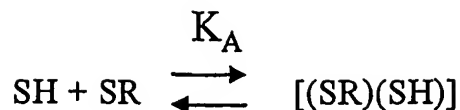
5 The invention also relates to diagnostic compositions containing CEST agents as defined above, mixed with a suitable vehicle.

All the molecules already described in US 5050609 and WO 00/66180 can be used as molecules with mobile protons, with particular preference for linear and macrocyclic polyamines such as cyclen (1,4,7,10-  
10 tetraazacyclododecane), polyaminoacids such as polyarginine, proteins such as human serum albumin, polysaccharides, polycyclodextrins, synthetic polymers (polyamidoamines, peramidated polyaminoacids, and dendrimers containing amide groups). Cyclen, polyarginine and albumin are particularly preferred.

Exchangeable protons may also belong to water molecules which,  
15 following interaction with the SH substrate and due to the nature of the adduct [(SR)(SH)], are "shifted" from the bulk water signal and are in suitable exchange conditions with it. This category of SH substances includes polysaccharide systems, especially systems which form supramolecular aggregates able to trap molecules of water and SR units. A further extension  
20 of this concept includes any system wherein SR and water molecules are trapped in the same compartment, and at the same time the water molecules (whose chemical shift is influenced by the SR unit) are in suitable exchange conditions with the bulk water. A non-exhaustive list of systems which can be used to obtain this compartmentalisation includes liposomes, nano- and micro-  
25 vesicular systems, protein cavities and cells.

The adducts according to the invention can be prepared by simple mixing of molecules having mobile protons with paramagnetic chelates, in suitable molar ratios which depend on the affinity between the substrate and

the SR unit:



This affinity can be expressed by the value of the thermodynamic  
5 constant of association ( $K_A$ ), which must be greater than 10. The adducts can then be isolated and purified by chromatographic techniques and/or freeze-drying, precipitation, evaporation and the like.

According to a first embodiment of the invention, the SR unit possesses chemical characteristics able to recognise the portion of the substrate molecule  
10 containing the mobile protons so as to significantly influence their chemical shift. For example, in the case of adducts between  $[\text{LnDOTP}]^{4-}$  (Ln is a lanthanide) and the preferred substrates cyclen, polyarginine and HSA, the interaction takes place through electrostatic bonds.

Other examples relating to the electrostatic interaction between  
15 substrate and SR unit are those in which the  $[\text{LnDOTP}]^{4-}$  is able to influence the chemical shift value of the mobile protons of diamagnetic molecules such as Co(III)-sarcophagine, Co(III)-(ethylenediamine)<sub>3</sub> and agmatine.

Recognition of the substrate-SR unit can also take place through hydrophobic (e.g. in the case of polycyclodextrins) or mixed interactions (e.g.  
20 in the case of proteins).

Alternatively, the SR unit can be trapped in polymer systems with high biocompatibility such as hydrogels or cross-linked proteins in which it can perform its effect towards the slow-exchange water molecules present in those systems.

25 Other systems of interest are those wherein differentiation between the pools of exchangeable protons takes place as a result of the effects of compartmentalisation.

According to this embodiment of the invention, the SR unit and SH

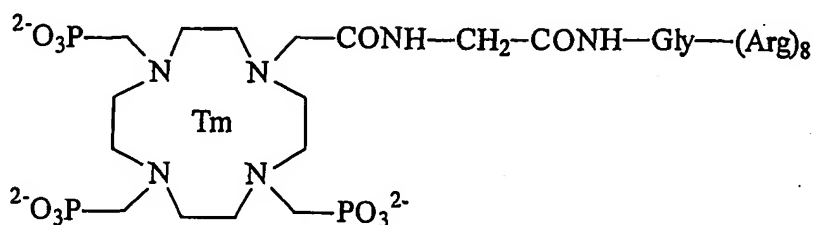
substrate can be compartmentalised in suitable biocompatible systems such as liposomes, nanoparticles, microemulsions and protein cavities (e.g. apoferritin), in which water (unlike the SR unit and the substrate) can be freely exchanged between the inside and outside of the compartment. In this way, the effect of the SR unit could also be performed by means of a simple variation in magnetic susceptibility able to influence the resonance frequency of the mobile protons of the substrate in the compartment without requiring their interaction, with the advantage of making the irradiation frequency of the mobile protons of the substrate almost constant.

10 If the SH-SR agent is compartmentalised in a system wherein the exchange rate of water between the inside and the outside of the compartment is very slow, and the quantity of water in the compartment containing the unit is sufficient to observe it in the MR image, the effect of saturation transfer could be considerably increased due to the increase in the local concentration of SR and in the substrate containing the mobile protons.

Alternatively, the shift agent could be chosen in order to influence resonance of the bulk water signal only. This effect would in any event increase the separation of the resonance frequencies between water and substrate, thus allowing irradiation of the mobile protons of the substrate. An example of this kind, unrelated to CEST, is reported by Otting et al. (J. Am. Chem. Soc., 1991, 113, 4363), who used Co(II) to "shift" the bulk water signal, thus allowing observation of the water molecules trapped in a site of protein BPTI, which the shift agent is unable to enter.

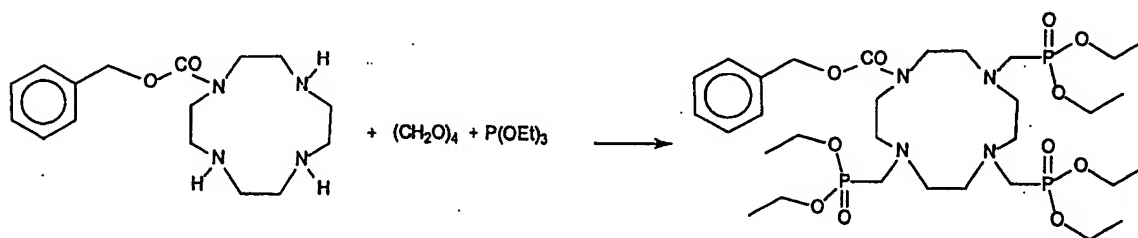
25 The SR unit and the SH unit can also be conjugated through a covalent bond so as to guarantee the same biodistribution of the two units *in vivo*. In this case, the effect of the SR unit is not performed intramolecularly towards the protons of the SH unit, but intermolecularly, through a mechanism of self-identification/self-aggregation.

A preferred compound in this category is the following:

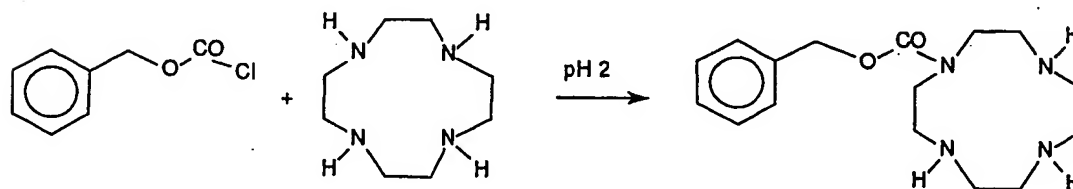


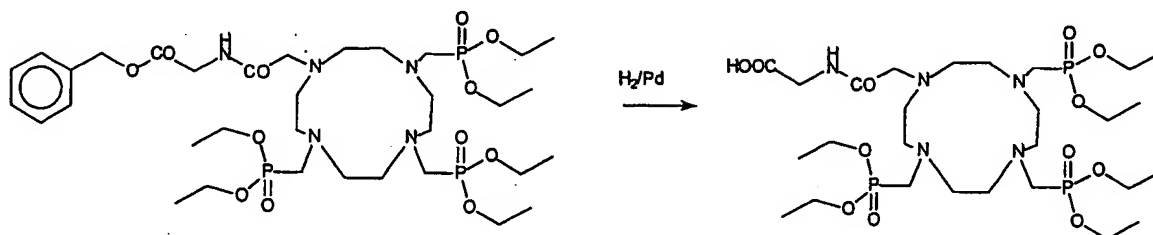
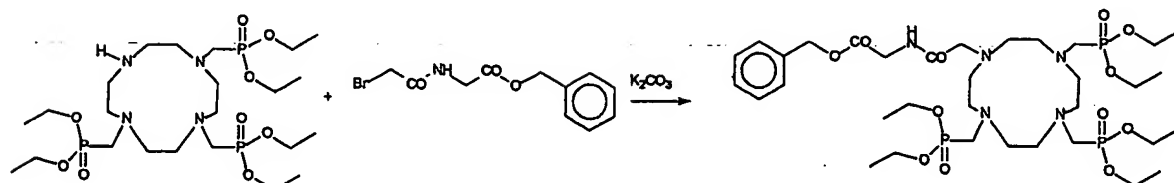
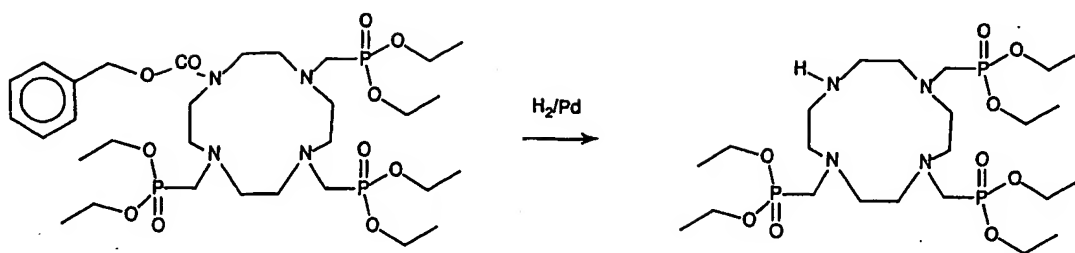
wherein intermolecular self-identification takes place between a negatively  
 5 charged SR unit of one molecule and the positive polyarginine chain of  
 another. In this compound, the choice of the number of arginine residues on  
 the side chain was dictated partly by the fact that the structural motif (Arg)<sub>8</sub>,  
 reminiscent of the peptide fragment 48-60 of virus HIV-1, is known for its  
 ability to be recognised and internalised by the cells (Suzuki et al., J. Biol.  
 10 Chem., 2002, 277, 2437).

This compound can be prepared according to the following synthesis  
 chart:

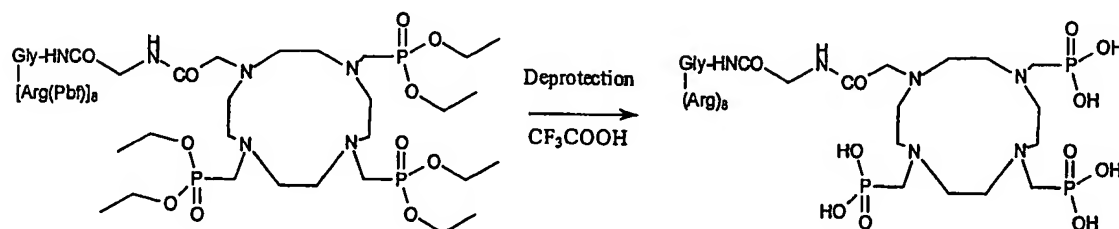
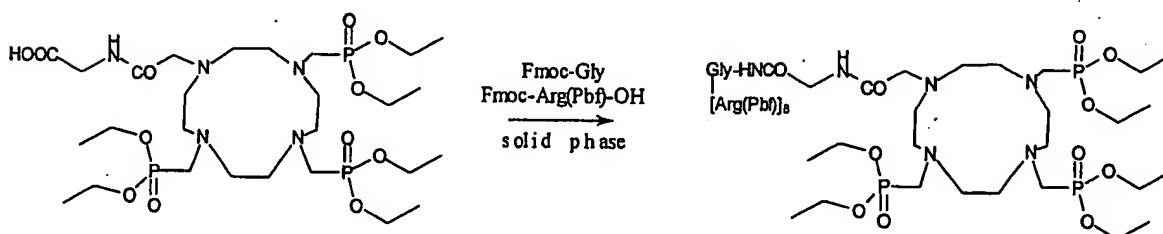


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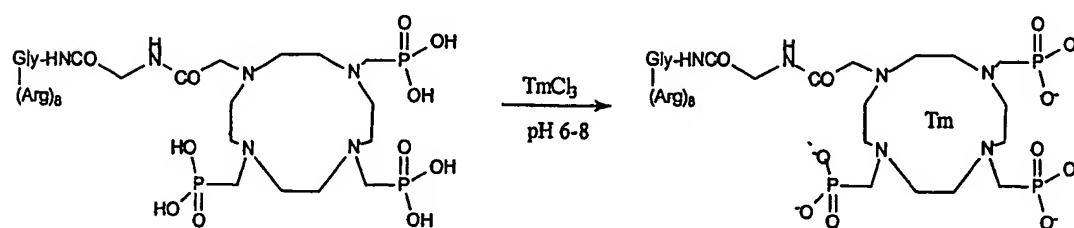




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For the proposed diagnostic uses, the agents according to the invention will be formulated in appropriate compositions suitable for parenteral administration.

The diagnostic compositions according to the invention can be used to  
5 determine the chemico-physical parameters of diagnostic interest such as temperature, pH, metabolite concentration, O<sub>2</sub> and CO<sub>2</sub> partial pressures and enzyme activity in a human or animal tissue or body by means of the CEST method. For this purpose, the preferable diagnostic composition should contain two magnetically different sets of mobile protons, whose transfer  
10 effect should preferably be different towards the chemico-physical parameter of interest. In this way, by using a ratiometric method (already described in WO 00/66180), it is possible to determine the diagnostic parameter independently of the local concentration of the diagnostic composition.

Another preferred embodiment relates to the possibility of using such  
15 systems to obtain information about endothelial permeability. For this purpose, conventional diagnostic agents (namely agents on T<sub>1</sub> or T<sub>2</sub> of water protons) (Min-Ying S. et al, Magn. Res. Med. 1998, 39, 259-269) are currently used with the DCE-MRI (DCE = Dynamic Contrast Enhanced) technique. The method involves the administration of diagnostic agents of different  
20 dimensions (as such, or after interaction with macromolecules like HSA) to evaluate the permeability of the capillaries. The quantitative evaluation of capillary permeability is carried out with a kinetic study which requires successive use of the different diagnostic agents. Using the CEST procedure, however, the extravasation of each SR unit (or each SH unit) can be evaluated,  
25 as the contrast can be "stimulated" by selective irradiation of the signals of the diagnostic system. On this basis, SR units of different sizes able to shift the signal of the mobile protons of substrate SH differently (e.g. by changing the paramagnetic metal) can be used. Identification of the species which pass

through the endothelium allows the kinetic study to be carried out after a single administration.

Alternatively, the same objective can be achieved by using the same SR unit and modulating the size and type of interacting substrate in order to vary the resonance frequency of its mobile protons. A similar result can be obtained by using compartmentalised systems with different dimensions wherein the diagnostic agent that passes through the endothelium can easily be identified with a suitable formulation of the solution contained in the chosen system (liposome, nanosphere, etc.).

The compositions preferably comprise an adduct according to the invention in molar concentrations of between about 0.001 and 1.0 M, in admixture with a suitable vehicle.

The compositions according to the invention can be suitably administered intravascularly (e.g. by the intravenous, intra-arterial or intraventricular route) or by the intrathecal, intraperitoneal, intralymphatic, intracavitary, oral or enteral route.

Preferred forms are sterile aqueous solutions or suspensions, ready for use or in the form of lyophilisate to be reconstituted with sterile solvent immediately before use. Depending on the specific diagnostic requirements, the dose can range between 0.01 and 0.5 mmol/kg of body weight.

The invention is described in greater detail in the examples below.

#### **Example 1**

CEST spectrum of a 0.1 mM solution of polyarginine (DP 227) at pH 6.5, 312 K, 7.05 T, irradiation time 10 s.

The spectrum (Figure 1) indicates the intensity of the NMR signal of the bulk water according to the irradiated frequency, expressed in ppm in relation to the water.

The two CEST peaks will be observed at around 2 ppm and 4 ppm off

the water, indicating the saturation transfer caused by irradiation of the guanidine protons and the amide backbone of the polymer respectively.

#### Example 2

CEST spectrum of a 0.1 mM solution of polyarginine (DP 227) at pH  
5 7.2, 312 K, 7.05 T, irradiation time 10 s.

The spectrum (Figure 2) demonstrates that the increase in the exchange rate of the polymer protons caused by the increase in pH leads to their coalescence with the bulk water signal.

#### Example 3

10 CEST spectrum of a 0.5 mM solution of polyarginine (DP 227) in the presence of 2.5 mM of [YbDOTP]<sup>4-</sup> at pH 6.5, 312 K, 7.05 T, irradiation time 4 s.

The spectrum (Figure 3) shows the effect of the SR unit on the resonance frequency of the substrate.

#### 15 Example 4

CEST spectrum of a 0.5 mM solution of polyarginine (DP 227) in the presence of 2.5 mM of [YbDOTP]<sup>4-</sup> at pH 7.2, 312 K, 7.05 T, irradiation time 4 s.

20 The spectrum (Figure 4) demonstrates that by increasing the pH of the solution, the effect of the SR unit allows measurement of a saturation transfer for guanidine protons by irradiating in the 5-10 ppm interval off the bulk water.

#### Example 5

25 CEST spectrum of a 0.25 mM solution of polyarginine (DP 227) in the presence of 3.5 mM of [DyDOTP]<sup>4-</sup> at pH 6.5, 312 K, 7.05 T, irradiation time 4 s.

The spectrum (Figure 5) indicates that the effect of the Dy(III) ion on the resonance of the guanidine mobile protons has the opposite mathematical

sign to the use of the Yb(III) ion.

#### Example 6

CEST spectrum of a 0.1 mM solution of polyarginine (DP 227) in the presence of 1.2 mM and 2.4 mM of [TmDOTP]<sup>4-</sup> at pH 7.2, 312 K, 7.05 T, irradiation time 4 s.

The spectrum (Figure 6) indicates the marked effect of this complex on the resonance frequency of the guanidine mobile protons, whose irradiation can be carried out in the 30-40 ppm interval (2.4 mM of SR).

#### Example 7

Dependence of saturation transfer (ST%) on a 6.25 μM solution of polyarginine (DP 227) according to the concentration of [TmDOTP]<sup>4-</sup> at pH 7.2, 312 K, 7.05 T, frequency of irradiation 8400 Hz off water, irradiation time 4 s.

The chart shows the considerable sensitivity of this system, which can be used to measure an ST effect of 5% at an SR unit concentration of about 40 μM.

#### Example 8

Dependence on pH of saturation transfer (ST%) of a 0.11 mM solution of polyarginine (DP 270) and 2 mM of [TmDOTP]<sup>4-</sup>, 312 K, 7.05 T, irradiation frequency 6000 Hz off water, irradiation time 4 s.

The effect considerably declines at an acid pH, mainly due to the reduction in exchange rate of the guanidine protons.

#### Example 9

Interaction between human serum albumin (HSA) and [TmDOTP]<sup>4-</sup> (312K, pH 7.4).

Figure 8 shows the saturation transfer measured by irradiating the frequencies of the NMR spectrum between 1.3 ppm and 10 ppm, for a solution of HSA 1 mM alone (□) and after addition of [TmDOTP]<sup>4-</sup> in the amount of

3mM (■). The frequency at which the maximum transfer is observed, attributable to irradiation of the guanidine protons of the Arg protein residues, shifts by about 2 ppm.

#### Example 10

5 Interaction between  $[\text{TmDOTP}]^{4-}$  and  $\text{Co(en)}_3^{3+}$ .

Figure 10 illustrates the dependency of the CEST effect (ST%) according to concentration for 1:1 solutions of  $[\text{TmDOTP}]^{4-}$  and  $\text{Co(en)}_3^{3+}$ , 298 K, pH 5.7, 7.05 T, frequency of irradiation 21,000 Hz off water, irradiation time 4 s. Under these experimental conditions, a 5% CEST effect  
10 has been obtained at an SR unit concentration of 0.1 mM. At physiological temperature and pH, the maximum effect is only 9% due to the high exchange rate of the primary amine protons.

#### Example 11

Interaction between  $[\text{TmDOTP}]^{4-}$  and Cyclen.

15 Figure 11 indicates the dependency of the CEST effect (ST%) according to the concentration ratio between Cyclen and  $[\text{TmDOTP}]^{4-}$  for a 4 mM solution of the latter, 312 K, pH 6, 7.05 T, frequency of irradiation 21,000 Hz off water, irradiation time 4 s. The maximum effect is observable for substrate/SR unit concentration ratios of about 3-4:1. At lower substrate  
20 concentrations the effect mainly declines because of the small number of protons irradiated, while at higher concentrations the effect mainly declines because of the increase in the free fraction of cyclen, with a consequent shift of the mobile proton signal to the diamagnetic area.

#### Example 12

25 Interaction between  $[\text{TmDOTP}]^{4-}$  and Cyclen.

Figure 12 illustrates the dependency of the CEST effect (ST%) on the concentration of  $[\text{TmDOTP}]^{4-}$ , maintaining a  $[\text{Cyclen}]/[\text{TmDOTP}]^{4-}$  concentration ratio of 3:1, 312 K, pH 6, 7.05 T, frequency of irradiation

21,000 Hz off water, irradiation time 4 s.

The chart shows the considerable sensitivity of this system, which can be used to measure an ST effect of 5% at an SR unit concentration of about 60  $\mu\text{M}$ .

5      **Example 13**

Interaction between  $[\text{TmDOTP}]^{4-}$  and Cyclen.

Figure 13 shows the dependency of the CEST effect (ST%) on pH for a 3:1 solution of cyclen (12 mM) and  $[\text{TmDOTP}]^{4-}$  (4 mM), 312 T, 7.05 T, irradiation frequency 21,000 Hz off water, irradiation time 4 s.

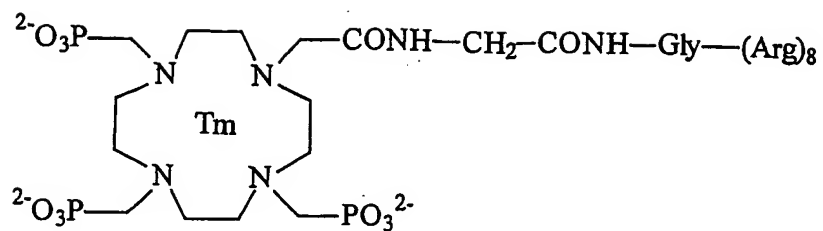
10      The transfer effect reflects the dependency of the amine proton exchange rate on the pH; in other words, it decreases when it is too slow (pH < 5) or when it is too fast, approaching the condition of coalescence (pH > 7).

**Example 14**

The histogram in figure 14 compares the values of ST% for a series of  
15      substrates interacting with the SR unit  $[\text{TmDOTP}]^{4-}$ , 312 K, pH 7.4, 7.05 T, irradiation time 4 s. In all cases the concentration of  $[\text{TmDOTP}]^{4-}$  is 4 mM, while that of the substrate varies according to the optimum concentration ratio previously evaluated. Triaza = 1,4,7-triazacyclononane 10:1; Hexaaza = 1,4,7,10,13,16-hexaazacyclooctadecane 2:1; Cyclen 3:1; Cyclam 2:1 and  
20      polyarginine 0.055:1.

## CLAIMS

1. Paramagnetic CEST agents comprising a substrate (SH) containing mobile protons bonded to a paramagnetic chelate (SR) containing a metal  
5 selected from iron (II) (high-spin configuration), iron (III), cobalt (II), rhodium (II), copper (II), nickel (II), cerium (III), praseodymium (III), neodymium (III), dysprosium (III), erbium (III), terbium (III), holmium (III), thulium (III), ytterbium (III) and europium (III).
2. Agents as claimed in claim 1, wherein the SH substrates are  
10 diamagnetic and selected from linear and cyclic polyamines, polyaminoacids, proteins, polysaccharides, polyamidoamines, peramidated polyaminoacids, dendrimers containing amide groups, polycyclodextrins, polysaccharides and alginates.
3. Agents as claimed in claim 2, wherein the substrate is selected from  
15 polyarginine, albumin and cyclen.
4. Agents according to any one of claims 1 to 3, wherein the paramagnetic chelate is  $[\text{LnDOTP}]^{4-}$ .
5. Agents according to any one of claims 1 to 4, compartmentalised in biocompatible systems selected from liposomes, nanoparticles,  
20 microemulsions and protein cavities.
6. Agents as claimed in any one of claims 1 to 5, wherein the bond between substrate and the chelate is of the electrostatic type, the apparent thermodynamic constant of SR-substrate association ( $K_A$ ) being greater than 10.
- 25 7. Agents according to any one of claims 1 to 5, wherein SH is bonded covalently to SR.
8. An agent as claimed in claim 7 with formula:



9. A diagnostic composition containing an agent as claimed in claims 1-8, in admixture with a suitable vehicle.



Figure 1

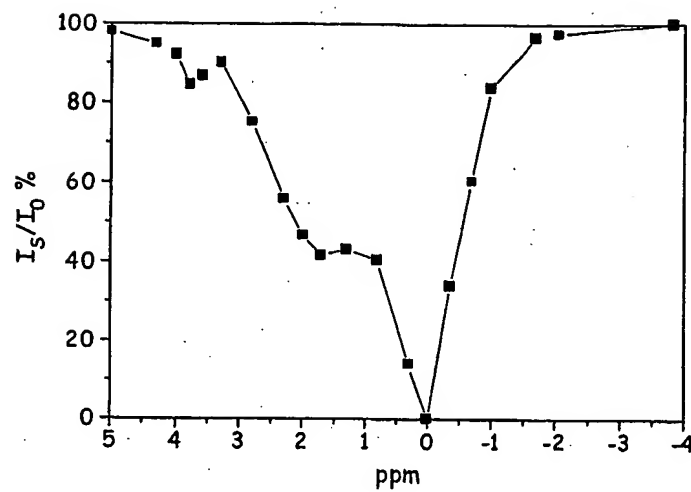


Figure 2

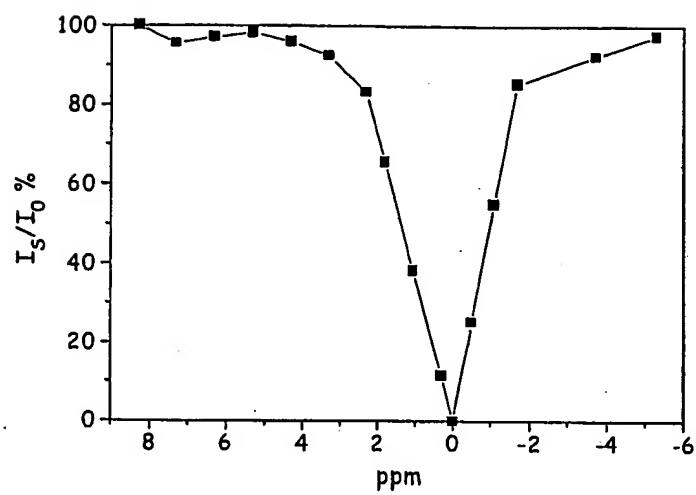
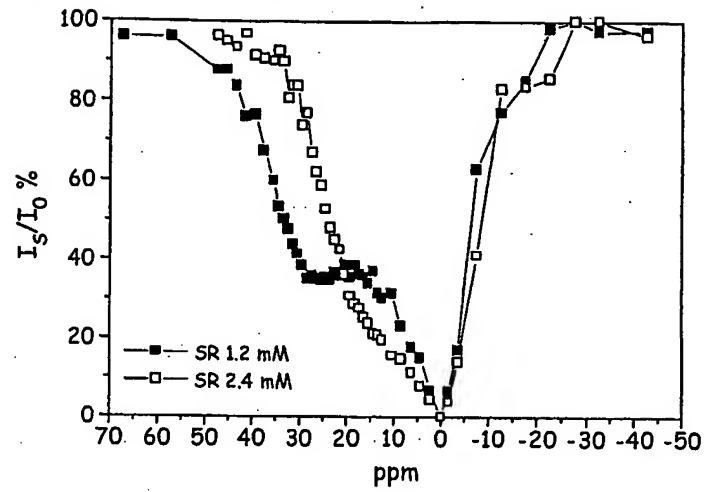
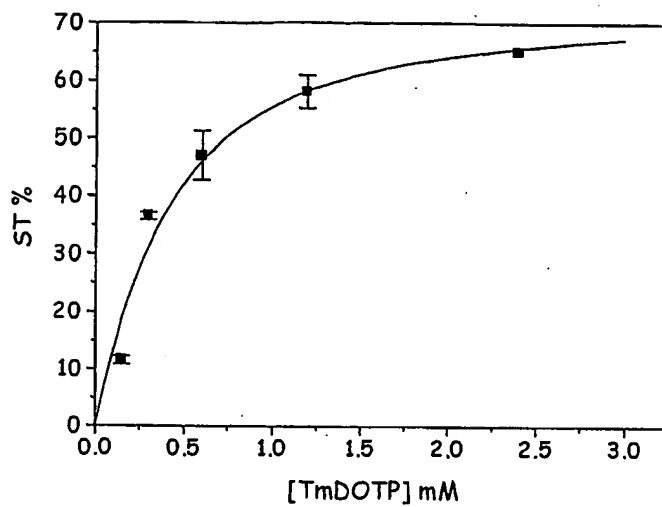


Figure 6



**Figure 7**

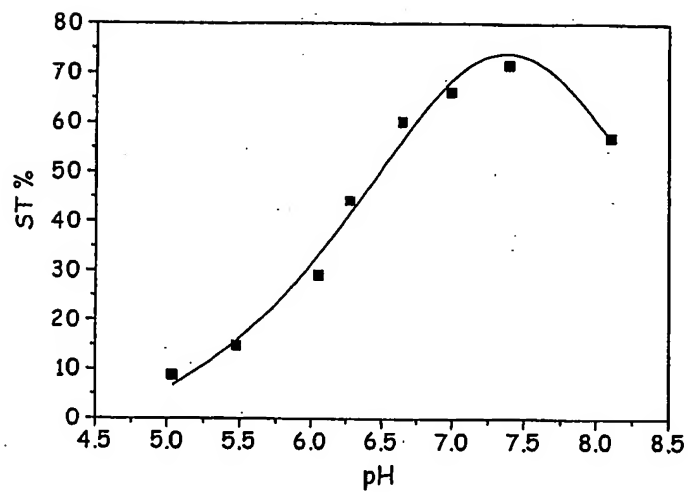
**Figure 8**

Figure 10

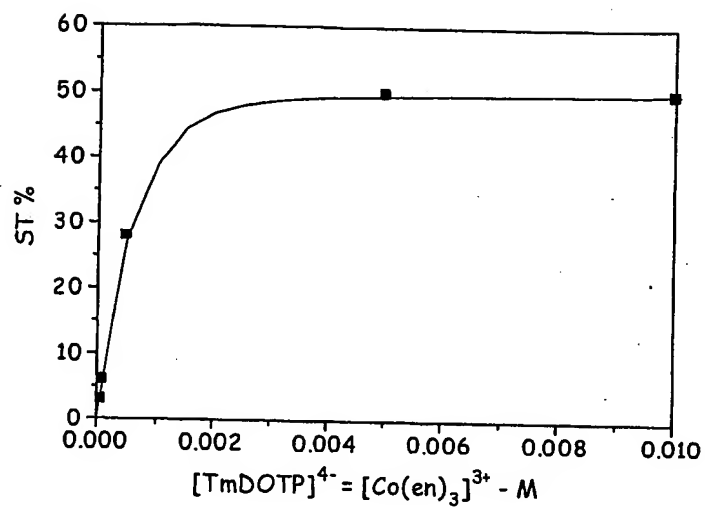
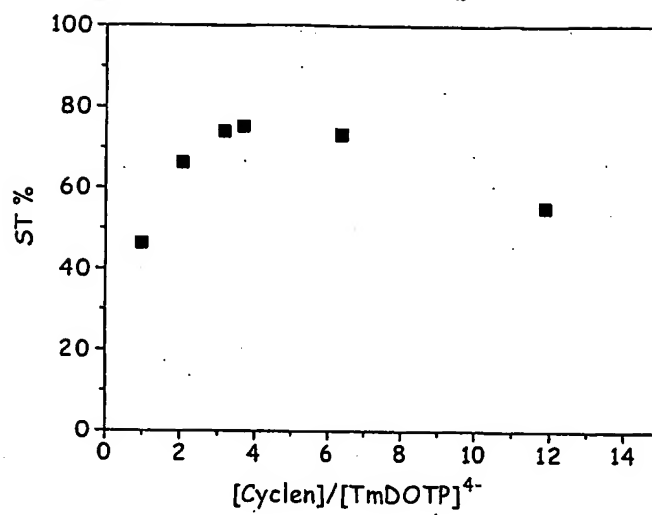


Figure 11



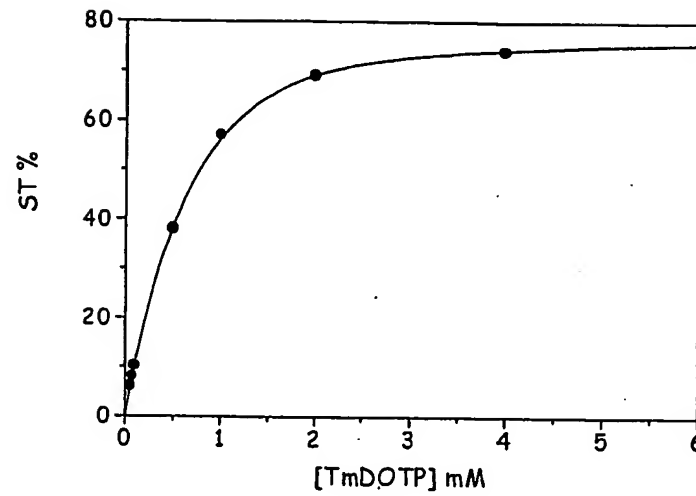
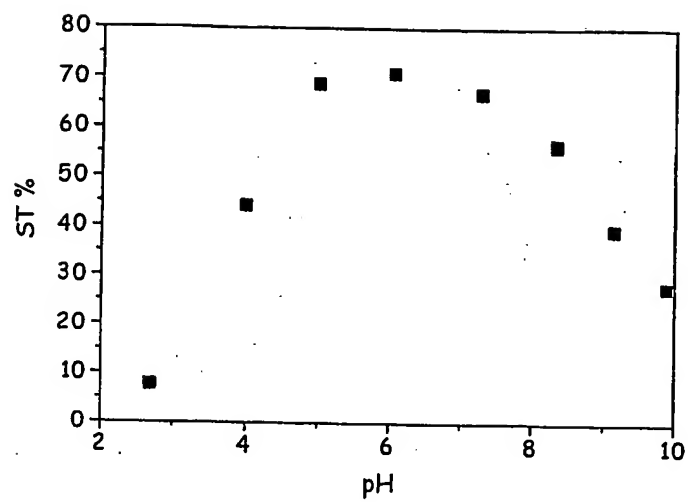
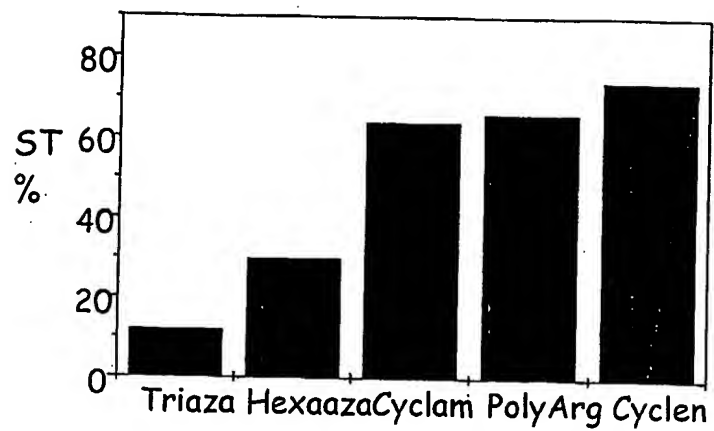
**Figure 12**



Figure 13



**Figure 14**

**A. CLASSIFICATION OF SUBJECT MATTER**  
**IPC 7 A61K49/08**

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

**IPC 7 A61K**

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

**EPO-Internal****C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96/38184 A (FRASER SCOTT ; JACOBS RUSSELL (US); MEADE THOMAS (US); CALIFORNIA I) 5 December 1996 (1996-12-05)	1,2,5,7, 9
Y	figures 3-6 claims 1,2,10,13 page 21, lines 5-8 page 45, line 2	1-9
X	WO 03/013617 A (SCHERING AG) 20 February 2003 (2003-02-20) page 13, lines 20-26 tables 1, compound, 68 claims 1, 11-13 * Page 17, compounds II and III * ----- -/--	1,2,7,9



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

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Date of the actual completion of the International search

**8 July 2004**

Date of mailing of the International search report

**10/09/2004**

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## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 1 106 186 A (MALLINCKRODT INC) 13 June 2001 (2001-06-13) paragraphs '0009! - '0012!, '0017!, '0020! examples 1,7 claims 1-3,8,13,14	1-3,6,9
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X	WO 02/43775 A (UNIV TEXAS) 6 June 2002 (2002-06-06) claims 10,14,17-19 page 13, line 30 - page 14, line 2 page 33, lines 8-10 page 35, lines 1-4 table 1	1,7,9
P,X	EP 1 331 012 A (BRACCO IMAGING S P A) 30 July 2003 (2003-07-30) See DOTAM-Gly paragraph '0092! claims 1-3,9	1,7,9
A	ZHANG S ET AL: "A NOVEL PH-SENSITIVE MRI CONTRAST AGENT" ANGEWANDTE CHEMIE. INTERNATIONAL EDITION, VERLAG CHEMIE. WEINHEIM, DE, vol. 38, no. 21, 2 November 1999 (1999-11-02), pages 3192-3194, XP000864995 ISSN: 0570-0833 * See compound 1 *figure 1	1,4,7,9
A	WO 99/55230 A (NAGY JAMES I ;TURLEY EVA ANNE (CA); HYAL PHARMA CORP (CA); MIKULIS) 4 November 1999 (1999-11-04) page 11, lines 1-32 page 22, line 5 - page 25, line 15 claims 3,11	6

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## C(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>AIME S ET AL: "PARAMAGNETIC LANTHANIDE(III) COMPLEXES AS PH-SENSITIVE CHEMICAL EXCHANGE SATURATION TRANSFER (CEST) CONTRAST AGENTS FOR MRI APPLICATIONS"</p> <p>MAGNETIC RESONANCE IN MEDICINE, ACADEMIC PRESS, DULUTH, MN, US, vol. 47, no. 4, 2002, pages 639-648, XP001151672</p> <p>ISSN: 0740-3194</p> <p>* See page 640; chart 1: DOTAM-Gly * table 1</p>	1,7,9
Y	<p>WO 00/66180 A (ALETRAS ANTHONY H ;US HEALTH (US); WARD KATHLEEN M (US); BALABAN R) 9 November 2000 (2000-11-09) cited in the application the whole document</p>	1-9
A	<p>WO 92/13572 A (DIATECH INC) 20 August 1992 (1992-08-20) page 13, line 35 claim 1</p>	8
X	<p>AIME S ET AL: "Non-covalent conjugates between cationic polyamino acids and GdIII chelates: a route for seeking accumulation of MRI-contrast agents at tumor targeting sites."</p> <p>CHEMISTRY (WEINHEIM AN DER BERGSTRASSE, GERMANY) 14 JUL 2000, vol. 6, no. 14, 14 July 2000 (2000-07-14), pages 2609-2617, XP002287529</p> <p>ISSN: 0947-6539</p> <p>page 2609, column 1</p> <p>page 2610, column 1</p> <p>See scheme 1, 2</p>	1-3,6,9
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